

1 5 10 15 20 25  
G P V C A E A S D V Y S P C M I A S T P P A P F S  
GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCCATGTATGATAGCTAGCACTCCTCCTGCTCCATTTC  
2670 2700 NheI 2730

<-----βC----->  
30 35 40  
D V T A V T F D L I N G K I T  
GACGTTACAGCAGTAACTTTTGACTTAATCAACGGCAAATAACT  
2760

FIGURE 1

(A)

S T Y S R N A V P N L R G D L Q V L A Q K V A R T L P  
 CTAGCACTTATAGTAGAAATGCTGTTCTAATTTGAGAGGAGATCTTCAAGTTTTGGCTCAAAAGGTTGCTCGGACTCTTC  
 GTGAATATCATCTTTACGACAAGGATTAACTCTCTCTAGAGTTCAAAACCGAGTTTCCAACGAGCCTGAGAAGGATC  
 BgIII

(B)

1 5 10 15 <-----  
 G P V C A E A S D V Y S P C M I A S T Y S R N A V P N  
 GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCCATGTATGATAGCTAGCACTTATAGTAGAAATGCTGTTCTAAT  
 2670 2700 NheI

-----> 20 25  
 L R G D L Q V L A Q K V A R T L P S T P P A P F S  
 TTGAGAGGAGATCTTCAAGTTTTGGCTCAAAAGGTTGCTCGGACTCTTCTAGCACTCCTCCTGCTCCATTTTCA  
 BgIII xNheI 2730

**FIGURE 2**

665050 2961050

O K V A R T L P S T P P A P F S D V T A V T F D L I  
 CAAAAGGTTGCTCGGACTCTTCTAGCACTCCTCTGCTCCATTTTCAGACGTTACAGCAGTAACCTTTGACTTAATC  
 GTTTTCCAACGAGCCTGAGAAGGATCGTGAGGAGGACGAGGTAAGTCTGCAATGTCGTCATTGAAAACCTGAATTAG  
 xNheI

**FIGURE 3**

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15 20 25 30 35  
P C M I A S T P P A P F S D V T A V T F D L I  
CCATGTATGATAGCTAGCACTCCTCCTGCTCCATTTTCAGACGTTACAGCAGTAACTTTTGACTTAATC  
2700 NheI 2730 \*



Site-directed Mutagenesis

15 20 25 30 35  
P C M I A S T P P A P F S D V T A V T F D L I  
CCATGTATGATAGCTAGCACTCCTCCTGCTCCATTTTCAGACGTTACAGCAGTAACTTTTGACTTAATC  
2700 NheI 2730 AatII 2760

FIGURE 4

665052 2964066

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(A)

S T D R P E G I E E E G G E R D R D R S D  
CTAGCACTGACCGCCCTGAGGGCATCGAGGAAGAGGGCGGTGAGCGCGATCGTGATCGTTCGGACGT  
GTGACTGGCGGGACTCCCGTAGCTCCTTCTCCCGCCACTCGCGCTAGCACTAGCAAGCC  
PvuI

(B)

1 5 10 15 <-----  
G P V C A E A S D V Y S P C M I A S T D R P E G I E  
GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCATGTATGATAGCTAGCACTGACCGCCCTGAGGGCATCGAG  
2670 2700 NheI

-----> 30 35  
E E G G E R D R D R S D V T A V T F D L I  
GAAGAGGGCGGTGAGCGGATCGTGATCGTTCGGACGTCACAGCAGTAACTTTTGAAGTAAATC  
PvuI AatII 2760

**FIGURE 5**

665050-23040600

(A)

S T P A T G I D N H R E A K L D  
 CTAGCACTCCTGCTACTGGAATCGATAATCATAGAGAAGCTAAATTGGACGT  
 GTGAGGACGATGACCTTAGCTATTAGTATCTCTTCGATTTAACC  
 ClaI

(B)

1 5 10 15 <-----  
 G P V C A E A S D V Y S P C M I A S T P A T G I D N  
 GGACCTGTTTGTGCTGAAGCCTCAGATGTGTATAGCCCA<sup>2670</sup>GTATGATAGCTAGCACTCCTGCTACTGGAATCGATAAT  
 NheI ClaI

-----> 30 35  
 H R E A K L D V T A V T F D L I  
 CATAGAGAAGCTAAATTGGACGTCACAGCAGTAAC<sup>2760</sup>TTTGACTTAATC  
 AatII

FIGURE 6

[illegible]

Q K V A R T L P D V T A V T F D L I  
 CAAAAGGTTGCTCGGACTCTTCCTGACGTCACAGCAGTAACTTTTGACTTAATC  
 GTTTTCCAACGAGCCTGAGAAGGACTGCGAGTGTCGTCATTGAAAACCTGAATTAG  
 AtaII

FIGURE 7

25  
P P S D  
CCATTTTCAGACGT  
GGTAAAGTC

FMDV-V

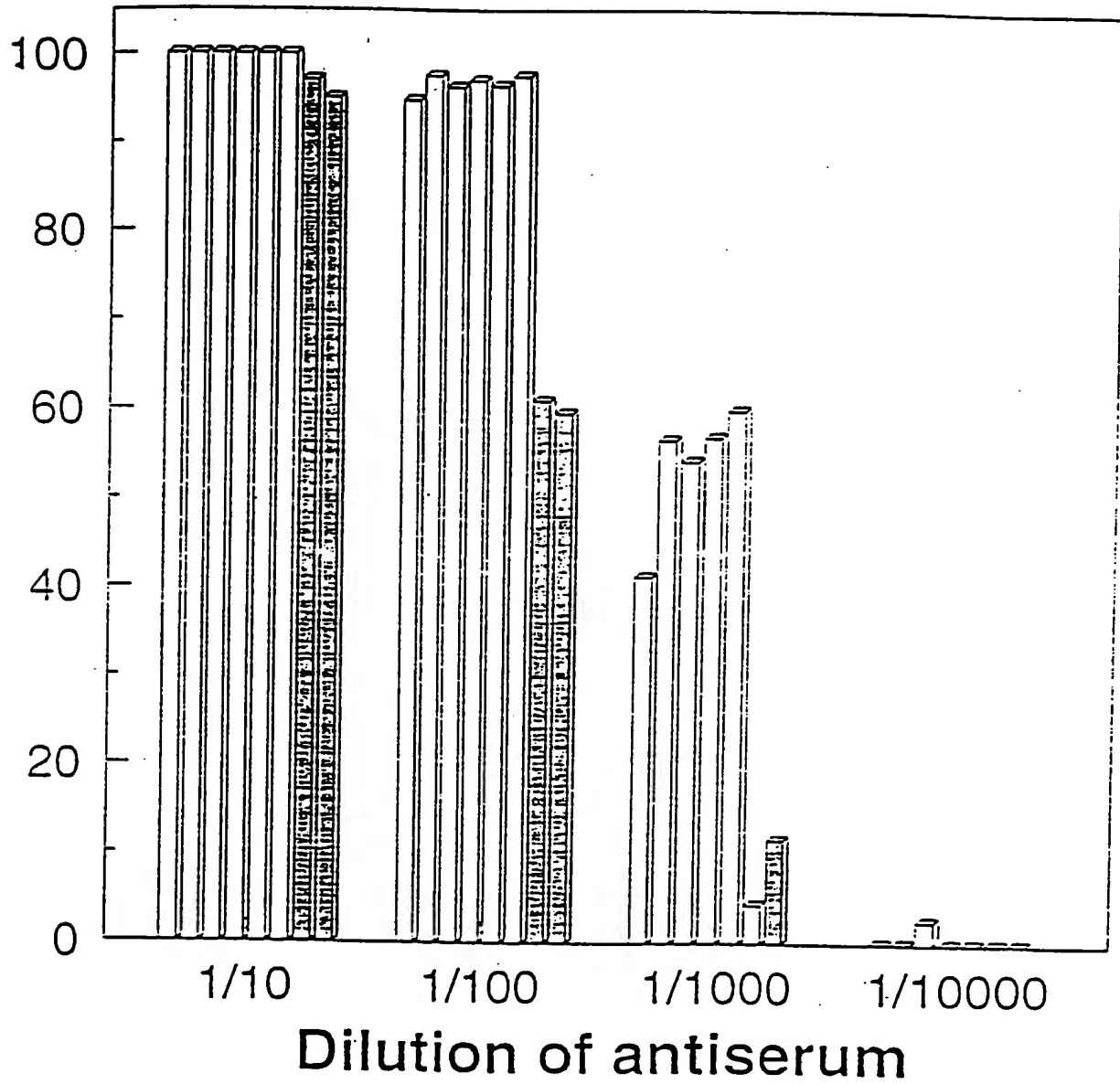
**HRV-II**

HIV-III

FIGURE 8



# % Neutralization



□ CPMV-HIV-I    ▨ wild-type CPMV

FIGURE 9

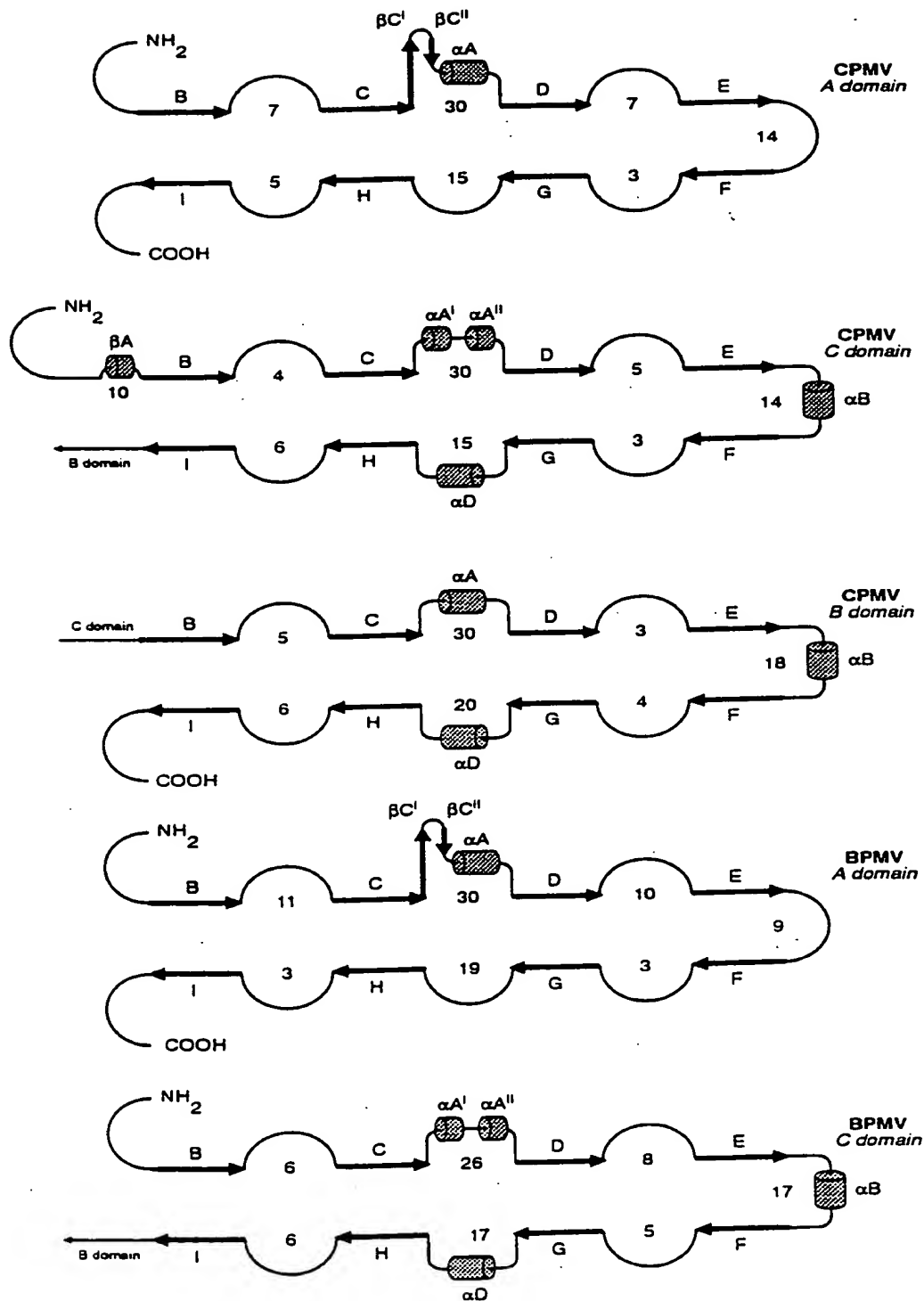


Figure 10

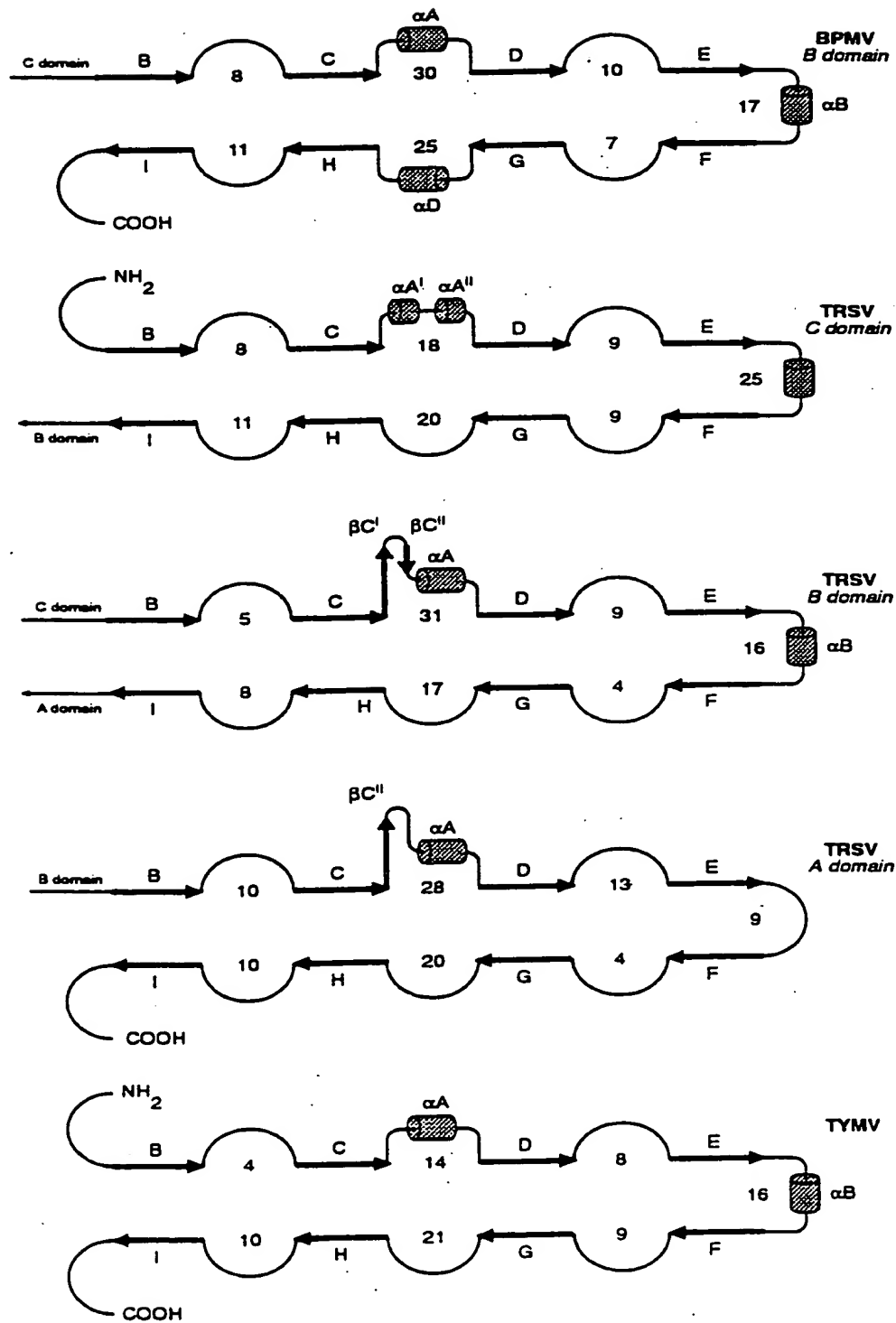


Figure 10

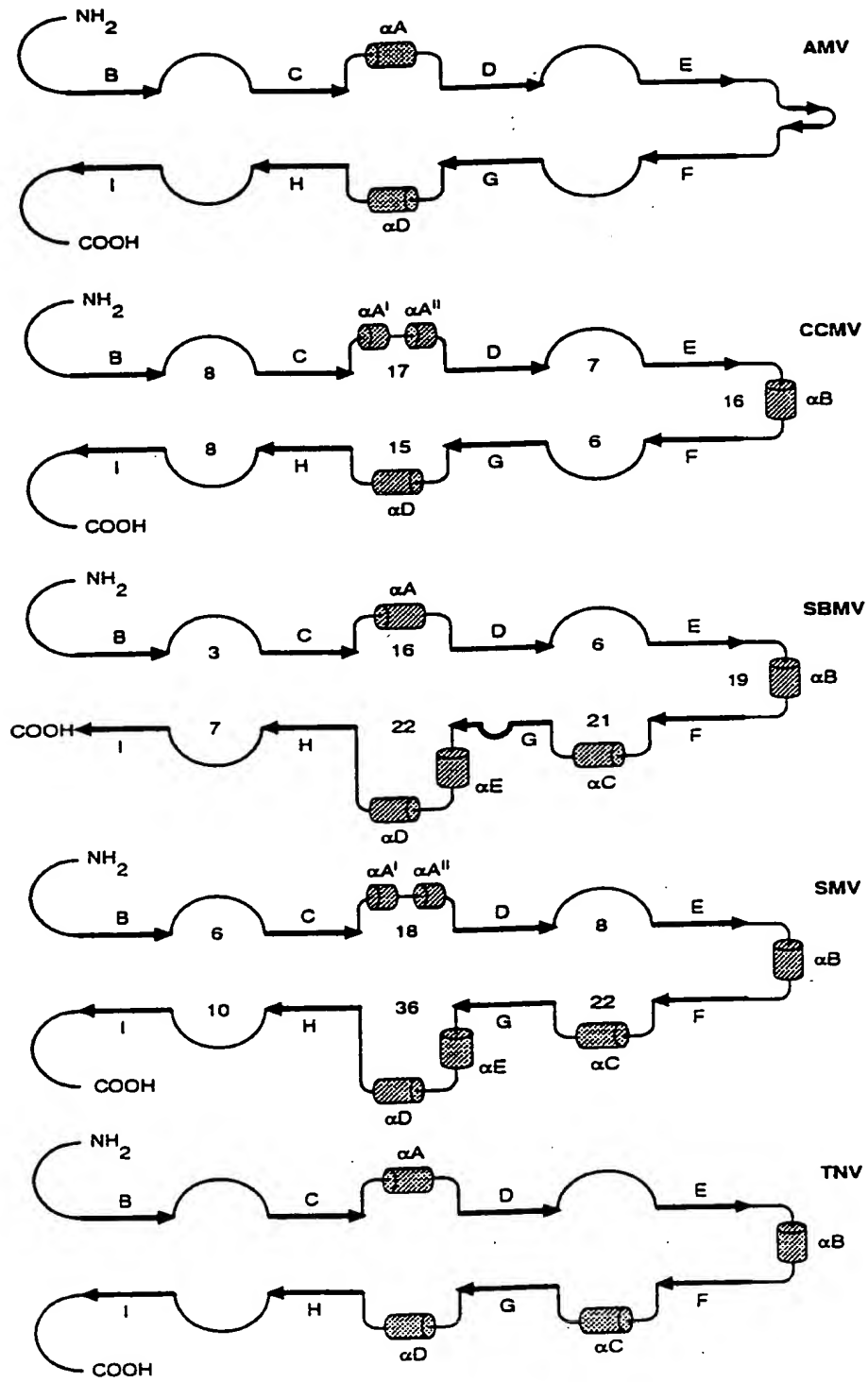


Figure 10

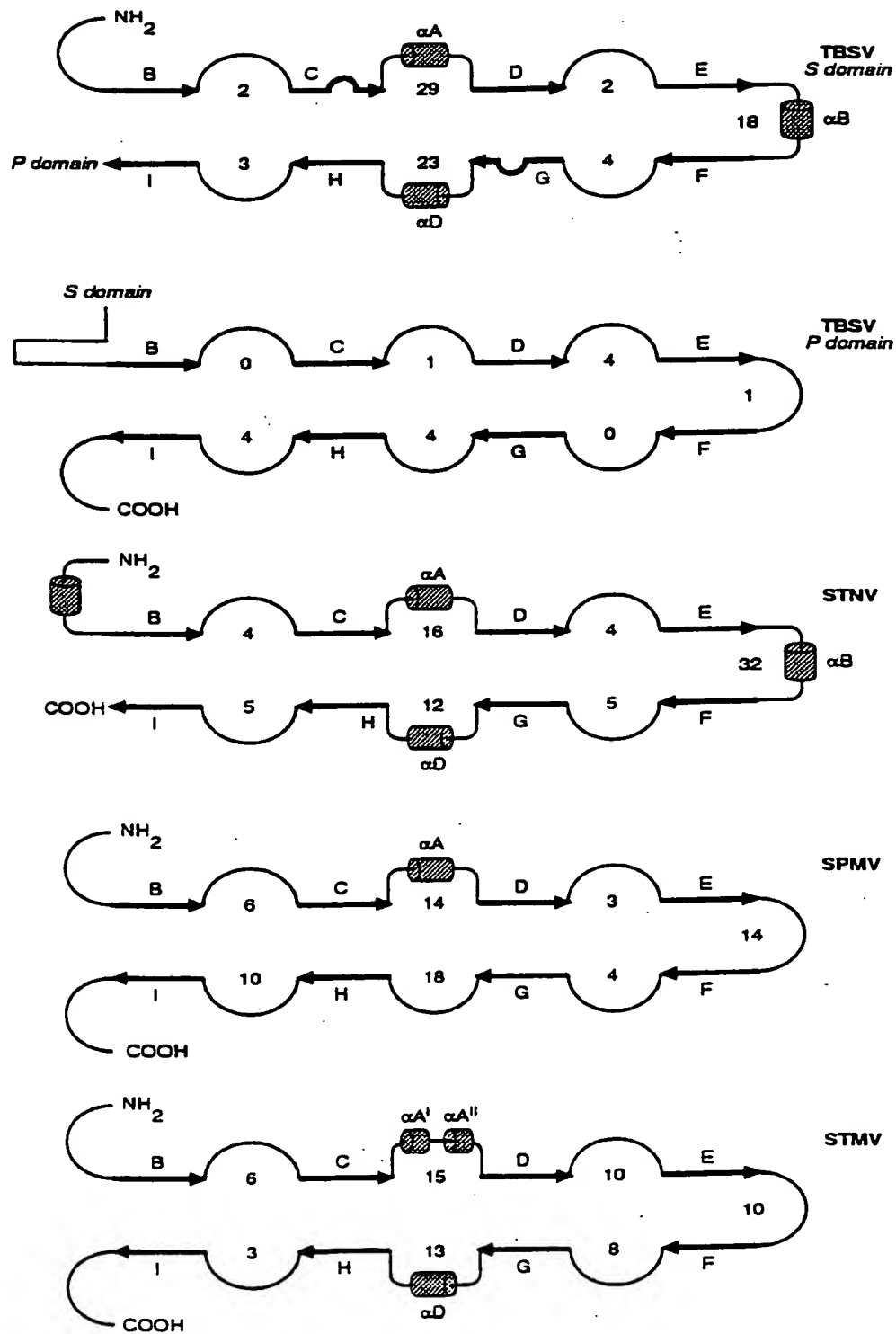


Figure 10

M E G G S S K T A V N T G  
ATGGAAGGAGGATCATCTAAGACTGCTGTGAACACTGGG

GTTAAC  
*Hpa* I

G V T S A P D T R P A P G S T A  
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT  
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GTT  
CAA

[illegible]

#####=====#####

$$\beta_H \quad \text{loop} \quad \beta_I$$
[illegible]

Figure 13

- (a) Sequence of LTSV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3954)

I A A A N S S I N I A S V G T L Y  
ATAGCCGCAGCTAACAGCTCCATAAACATAGCTAGTGTGGGTACTCTTTAT  
↓ ↓  
CTGCAG GGTACC  
*Pst*I *Kpn*I

- (b) Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations.

G V T S A P D T R P A P G S T A  
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT  
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GCTAACAGC  
ACGTCGATTGTCG

GCTAACAGCTCC  
ACGTCGATTGTCGAGG

GCTAACAGCTCCATA  
ACGTCGATTGTCGAGGTAT

GCTAACAGCTCCATAAAC  
ACGTCGATTGTCGAGGTATTTG

GCTAACAGCTCCATAAACATA  
ACGTCGATTGTCGAGGTATTTGTAT

GCTAACAGCTCCATAAACATAGCT  
ACGTCGATTGTCGAGGTATTTGTATCGA

TCCATAAACATAGCTAGTGTGGGTAC  
AGGTATTTGTATCGATCACACC

ATAAACATAGCTAGTGTGGGTAC  
TATTTGTATCGATCACACC

AACATAGCTAGTGTGGGTAC  
TTGTATCGATCACACC

ATAGCTAGTGTGGGTAC  
TATCGATCACACC

GCTAGTGTGGGTAC  
CGATCACACC

AGTGTGGGTAC  
TCACACC



Figure 14

Lipman-Pearson alignment of RCNMV and TBSV coat protein sequences.

## Lipman-Pearson Protein Alignment

Ktuple: 2; Gap Penalty: 4; Gap Length Penalty: 12

Seq1(1>389)	Seq2(1>340)	Similarity	Gap	Gap	Consensus
tbsvtbs.PRO	rcnmvdia.PRO	Index	Number	Length	Length
(64>387)	(8>338)	26.9	4	7	331

```

      70      80      90     100     110     120
KKQQMINHVGGTGGAIMAPVAVTRQLVGSKPKFTGRTSGSVTVTHREYLSQVNNSTGFQV
K.:Q. . . . T . . . . VA: . . . . . . . . . . H . . . V .S. . . .
KSKQRSQPRNRTPTNTSVKTVAIPFAKTQIIKTVNPPPKPARGILHTQLVMSVVGSVQMRT
  ^10      ^20      ^30      ^40      ^50      ^60
      130     140     150     160     170     180
NGGIVGNLLQLNPLNGTLFSWLPAIASNFDDQYTFNSVVLHYVPLCSTTEVGRVAIYFDKD
N.G . . . . LNP N :LF: L: A:N:D Y : . . . . L:YVPL . . . GRVA: .D D
NNGKSNQRFRLNPSNPALFPTLAYEAANYDMYRLKKLTTRYVPLVTVQNSGRVAMIWDPD
  ^70      ^80      ^90     100     110     120
      190     200     210     220     230     240
SEDPEPADRVELANYSVLKETAPWAEAMLRVPTDKIKRFCDDSSSTDHKLIDLGLGIAT
S:D:.P..R E:..YS .TA . . . L :P:D: RF .D::T D:KL:D:GQL :.T
SQDSAPQSRQEI SAYSRSVSTAVYEKCSLTIPADNQWRFVADNTTVDRKLVDFGQLLFVT
  ^130     ^140     ^150     ^160     ^170     ^180
      250     260     270     280     290     300
YGGAGTNAVGDIFISYSVTLYFPQPTNTLLSTRRLDLAALVTASGPGYLLVSR---TAT
.:G:.. .GDIF:..V.: PQPT:..: . :DL:G:L: .GP:YL: . :T:..
HSGSDGIEITGDI FLDCVEFEKGPQPTASIVQKTVIDLGGTLTSFEGPSYLMPPDAFITSS
  ^190     ^200     ^210     ^220     ^230     ^240
      310     320     330     340     350
VLTMTFRATGTFVISGTYRCLTATTLGLAG--GVNVNSITVVDNIG-TDSAFFINCTVSN
.: . . :GT:..: . C T:..:..:G .: .: .: .: .: .S F:..V :
SFGLFVDVAGTYLLTLVVTCSTTGSVTVGGNSTLVGDGRAAYGSSNYIASIVFTSSGVLS
  ^250     ^260     ^270     ^280     ^290     ^300
      360     370     380
LPSVVTFT-STGITSATVHCVRATRQNDVSL
.: V F: S:G:..: .: R .: N. L
TTPSVQFSGSSGVSRVQMNICRCKQGNTFIL
  ^310     ^320     ^330

```

Figure 15

Beta plot - Chou-Fasman

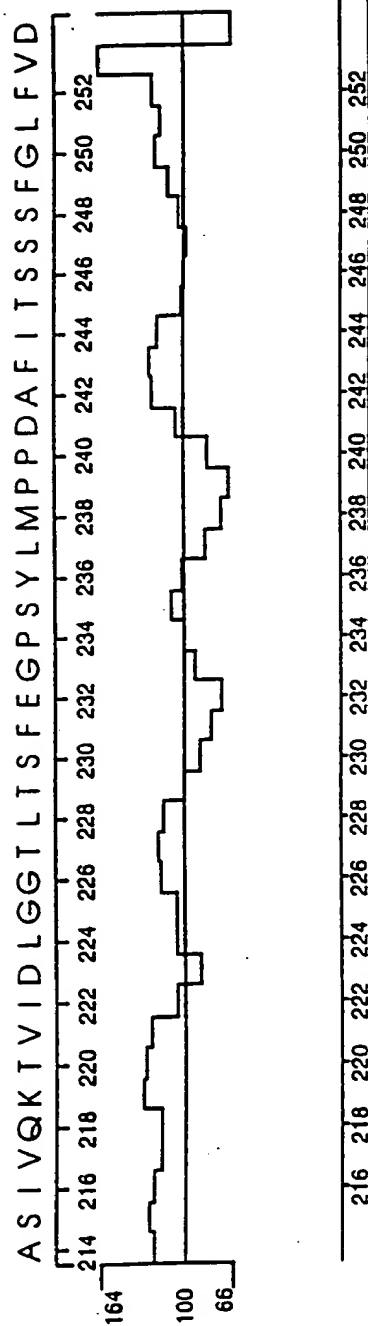


Figure 16

		220	230	240
AA		ASIVQKYVIDLGGTLTSFEGPSYLMPP		
PHD sec		HHHHHEEEEE	EEEE	EEEE
Rel sec		145432244525515625586487624		
detail :				
prH sec		4666553211111000000000000000		
prE sec		101123456632246752212688753		
prL sec		422221112246642237787311246		
subset : SUB sec		..H.....E.LL.EE.LLLL.EEE..		

## Abbreviations :

AA : amino acid sequence

H : helix

E : extended (sheet)

blank : other (loop)

PHD : Profile network prediction HeiDelberg

Rel : Reliability index of prediction (0-9)

prH : probability for assigning helix

prE : probability for assigning strand

prL : probability for assigning loop

SUB : a subset of the prediction, for all residues with an average expected accuracy of &gt;82%

Figure 17

(a) Sequence of RCNMV Coat Protein Spanning The Potential Insertion Site With Introduced Base Changes and New Restriction Sites: (sequence starts at nt 3070)

S I V Q K T V I D L G G T L T S F  
AGCATCGT**A**CAGAAA**A**CTGTAATTGATCTCGGTGGGAC**A**CTCACTTCTTTC  
          ↓      ↓                                  ↓      ↓  
          GTGCAC                                  GTTAAC  
          *Apa*LI                                  *Hpa*I

(b) Series of Sequences to be Inserted Between the Restriction Sites to Insert the MUC1(16) Epitope at Various Locations

G V T S A P D T R P A P G S T A  
GGTGTTACTTCTGCTCCTGATACTAGACCTGCTCCTGGTTCTACTGCT  
CCACAATGAAGACGACCACTATGATCTGGACGAGGACCAAGATGACGA

GAAAACTGTA  
ACGTCTTTTGACAT

GAAAACTGTAATT  
ACGTCTTTTGACATTAA

GAAAACTGTAATTGAT  
ACGTCTTTTGACATTAATA

GAAAACTGTAATTGATCTC  
ACGTCTTTTGACATTAAGTAGAG

GAAAACTGTAATTGATCTCGGT  
ACGTCTTTTGACATTAAGTAGAGCCA

GAAAACTGTAATTGATCTCGGTGGG  
ACGTCTTTTGACATTAAGTAGAGCCACCC

ATTGATCTCGGTGGGACGTT  
TAACTAGAGCCACCCTGCAA

GATCTCGGTGGGACGTT  
CTAGAGCCACCCTGCAA

CTCGGTGGGACGTT  
GAGCCACCCTGCAA

GGTGGGACGTT  
CCACCCTGCAA

GGGACGTT  
CCCTGCAA

ACGTT  
TGCAA

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Figure 18

(a) Nucleotide and amino-acid sequence of the C-terminal region of the coat protein of TRV :

S T P A S G S G A T P P A S G G A V R P N P \*  
 1135 CGTGGACTCCGGCCTCGGGGGAAGTGGTCAACACACCTCCTGCGAGTGGGGTGCTGTGCTGATGTCGTCAAATCAAACCTTTAAGGACCTT<sup>1230</sup>  
 ↑  
 Sa/I PpuMI

(b) Series of sequences to be inserted between the Sal I and PpuMI restriction sites to create C-terminal deletions :

S T P A S G S G A T P P A S G G A \*  
 TCGACTCCGGCCTCGGGGGAAGTGGTCAACACACCTCCTGCGAGTGGGGTGCTTGTGATGTCGTCAAATCAAACCTTTAAGG  
 GAGGCCGGAGCCCCCTTCACCACGTTGTGGTGGAGGACGCTCACCCACGAACCTACAGCAGTTTAGTTTGGAAATTCCTG

S T P A S G S G A T P P P \*  
 TCGACTCCGGCCTCGGGGGAAGTGGTCAACACACCTCCTTGTGATGTCGTCAAATCAAACCTTTAAGG  
 GAGGCCGGAGCCCCCTTCACCACGTTGTGGTGGAGGAACCTACAGCAGTTTAGTTTGGAAATTCCTG

S T P A S G S G \*  
 TCGACTCCGGCCTCGGGGGAAGTGGTCAATGTCGTCAAATCAAACCTTTAAGG  
 GAGGCCGGAGCCCCCTTCACCACCTACAGCAGTTTAGTTTGGAAATTCCTG

S T P A \*  
 TCGACTCCGGCCTGATGTCGTCAAATCAAACCTTTAAGG  
 GAGGCCGGACTACAGCAGTTTAGTTTGGAAATTCCTG

S T \*  
 TCGACTTGTGATGTCGTCAAATCAAACCTTTAAGG  
 GAACACAGCAGTTTAGTTTGGAAATTCCTG